METHOD AND COMPOSITIONS USEFUL IN PREVENTING EQUINE INFLUENZA

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of U.S. patent application Ser. No. 747,020, filed 20 June 1985 now allowed U.S. Pat. No. 4,361,191.

TECHNICAL FIELD

The invention relates to immunizing horses against infection by influenza virus. More particularly, the invention relates to use of vaccinia-carried immunogens and synthetic peptide vaccines useful for this purpose.

BACKGROUND ART

Equine influenza is a highly contagious respiratory infection engendered in horses by equine influenza virus (EIV). While the disease has low mortality, the economic impact is often great due to deterioration of the subject's performance (Mumford, J. A., et al, *Equine Vet J* (1980) 12: 3-9). Current immunization techniques use inactivated or killed EIV. These procedures are characterized by undesirable side effects, and the immunity conferred appears in some cases to last for no more than three or four months. In short, there is no entirely satisfactory vaccine available to prevent the spread of this disease.

Two different serotypes, which are members of the influenza A myxovirus group, have been identified as causative agents of this disease: A/equine/Prague/1/56 (Sovinova, O., et al, *Acta Virol* (English ed.) (1958) 2: 52-61) (designated herein EVI-A1) and A/equine/-Miami/1/63 (Waddell, G. H., et al, *J Am Vet Med Assoc* (1963) 143: 587-590) (designated herein EIV-A2). These strains share the characteristics associated with influenza viruses in general, including those viruses responsible for human influenza. Most importantly, the immunological characteristics of influenza virions appear to reside primarily in two virally encoded glycoproteins, the hemagglutinin protein (HA) and the neuraminidase protein (NA), both of which are embedded in the membranous envelope which comprises the outer layer of the virus. These proteins attach themselves to the outer membrane of infected cells. Twelve subtypes of HA (H1-H12) and nine subtypes of NA (N1-N9) have been defined using serological cross-reactivity. Thus, all influenza virus isolates carry a parenthetical description (HxNy) corresponding to the subclasses carried. The two equine strains are designated H7N7 (for EIV-A1) and H3N8 (for EIV-A2). (The designation H3 now is used to include both human H3 and former equine HEq2, although these are not identical proteins. H7 is now used to include both avian Hav7 and former HEq1; these, too, are not identical. N7 is former NEq1 and N8 is former NEq2. (Melnick, J., *Prog Med Virol* (1980) 26: 214-232).)

Both HA and NA are involved in the disease process. HA functions in attachment to host membrane and penetration into the host's cells; immunization in subject experimental animals with HA alone elicits neutralizing antibodies and protection from the disease. NA functions in cell-to-cell transmission, and antisera raised against NA attenuate the disease and decrease the spread of the disease from cell to cell. (Schulman, J. L., in *The Influenza Viruses and Influenza* (1975), E. D. Kilbourne, ed, New York/London: Academic Press, pp. 373-393.)

Human influenza virus and the design of vaccines to protect against it have received considerable attention. Synthetic peptides designed to correspond to a putative antigenic epitope on human HA glycoproteins have been attached to carrier proteins and used to immunize mice against infection with the human influenza virus of the same strain (U.S. Pat. No. 4,474,757). The peptides synthesized were apparently designed based on the sequence of fragments generated by CNBr digestion of the hemagglutinin protein. Synthetic peptide-carrier vaccines have also been suggested as a general approach to protection against viral infection (Brown, F., *Ann Rev Microbiol* (1984) 3: 221-235).

An alternative approach to vaccine compositions which has been suggested recently utilizes the vaccinia virus as a carrier. Mackett, M., et al, *J. Virol* (1984) 49: 857-864 describes this general method. Breifly, vaccinia is a large (187 kb) double-stranded DNA virus which replicates in the cytoplasm of infected cells. It is noninfectious when deproteinized, as it carries its own enzymes for transcription and cannot utilize the machinery of the host cell for this purpose. Vaccinia virus per se was used as the original smallpox vaccine, and is highly desirable as a vaccine carrier because of its low cost and ease of propagation. Freeze-dried vaccinia virus used against smallpox could be mass produced for as little as two cents per dose, while other subunit vaccines, for example, those against hepatitis B, cost approximately $100 per course of immunization. There are other advantages as well. Vaccinia stimulates both the humoral antibody and cell-mediated immunity systems of the subject. The freeze-dried vaccine is stable without refrigeration and is generally potent after a single inoculation. It is also easy to administer under nonsterile field conditions (Smith, G. L., et al, *Biotechniques* (1984) November/December: 306-312). Because of these advantages, vaccinia has been used as a carrier for antigenic proteins of hepatitis B and herpes simplex (Paoletti, E., et al, *Proc Natl Acad Sci* (USA) (1984) 81: 193-197), rabies (Wiktor, T. J., et al, *Proc Natl Acad Sci* (USA) (1984) 81: 7194-7198), and human influenza hemagglutinin (Panicali, D., et al, *Proc Natl Acad Sci* (1983) 80: 5364-5368; Smith, G. L., et al, *Proc Natl Acad Sci* (1983), 80: 7155-7159).

One reason these techniques have not been extensible to equine influenza is the absence of sufficient information on the HA and NA proteins of these viruses. This deficiency is remedied by the present invention, which provides complete genomic and amino acid sequences for the four relevant surface proteins characterizing EIV. In addition, the invention provides properly designed vectors and peptides based on these sequences useful in vaccine compositions.

DISCLOSURE OF THE INVENTION

The invention provides complete genetic sequences encoding the four glycoproteins which characterize the two identified serotypes of EIV. The invention further provides vaccine compositions which are grounded in this genetic information. One class of vaccine compositions comprises modified vaccinia engineered to express the HA and NA surface glycoproteins of the equine infective agents. Another class of compositions comprises synthetic peptide sequences designed to correspond to the antigenic determinants of the HA proteins. These synthetic peptides can be size enhanced, such as